

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Rui Sousa, et al.
Serial No.: 09/965,346
Filed: September 27, 2001
For: METHODS FOR USING MUTANT RNA
POLYMERASES WITH REDUCED
DISCRIMINATION BETWEEN NONCANONICAL
AND CANONICAL NUCLEOSIDE
TRIPHOSPHATES
Group Art Unit: 1634
Examiner: A. Chakrabarti

MARKED UP VERSION OF THE CLAIMS

41. (Amended) A method for determining sequence of a nucleic acid molecule, comprising the steps of:

a) synthesizing a nucleic acid molecule [de novo] from a RNAP promoter sequence in a reaction mixture containing a mutant T7-type RNA polymerase in each of four separate reactions, wherein said mutant T7-type RNA polymerase, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122, has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydroxy or a

hydrogen or a fluorine at the 2'-position, and further comprising a ddNTP, such that each of the four separate reactions forms a plurality of reaction products of differing length, the length of said reaction products indicating the positions or the type of base corresponding to the incorporated ddNTP, and

b) evaluating the reaction products so that the sequence of the template molecule may be deduced.

55. (Amended) A method for determining sequence of a nucleic acid molecule, comprising the steps of:

a) synthesizing a nucleic acid molecule [de novo] from a RNAP promoter sequence in a reaction mixture containing a mutant T7-type RNA polymerase, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122, in each of four separate reactions, wherein said mutant T7-type RNA polymerase has a reduced, discrimination between canonical and non-canonical nucleoside triphosphates, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydrogen or a

- fluorine at the 3'-position, and further comprising a rNTP;

b) treating the nucleic acid products of the reactions so as to bring about hydrolysis of the rNTP has been incorporated, whereby a plurality of reaction products of differing length are formed, the length of said reaction products indicating the positions of the type of base corresponding to the incorporated rNTP; and

b) evaluating the reaction products so that the sequence of the template molecule may be deduced.